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FACTORS INFLUENCING THE ROTTING OF CONIFEROUS LOGS

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Effect of bluestain fungi on southern pines attacked by bark beetles[1].

By

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Silviculturist[3], Appalachian Forest Experiment Station, Forest Service, United States Department of Agriculture[4].

With 6 Figures.

Contents: Introduction. — Historical review. — Bark beetles and their relation to bluestain. — *Ceratostomella pini* and *Ceratostomella ips* in culture. — *Ceratostomella pini* and *Ceratostomella ips* in sapwood of pines. — Inoculation of pines with *Ceratostomella pini* and *Ceratostomella ips*: 1929 and 1930 experiments. Discussion of inoculation results. — Experiments on water relations of pines: Comparison of unattacked pines with those infested with *Dendroctonus frontalis*. Comparison of uninoculated trees with those inoculated with Ceratostomella. — Experiments on ascent of dye in pine saplings: Comparison of living and killed saplings. Comparison of uninoculated saplings with those inoculated with Ceratostomella. Comparison of uninoculated saplings with those inoculated with Trichoderma. — Comparison of tori in stained and unstained wood. — Discussion. — Summary. — Literature cited.

Introduction.

The southern pine beetle *(Dendroctonus frontalis* Zimm.) is a particularly destructive insect in the pine forests of the South. The constant presence of bluestain fungi in trees successfully attacked by the southern pine beetle; the fact that bluestain is found in the water-conducting tissues of the xylem, whereas the beetles are chiefly confined to the phloem and cambium regions; and the extremely rapid death of infected trees, apparently due to a deficiency of water, have focused attention on

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[1]) Received for publication December 1932; issued September 1934.

[2]) This paper was presented in partial fulfillment of the requirements for the degree of doctor of philosophy in the University of Minnesota.

[3]) The investigations reported in this paper were made while the writer was Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[4]) The writer is indebted to Dr. Carl Hartley of the Division of Forest Pathology, U. S. Department of Agriculture; to members of the Divion of Forest Insects, Bureau of Entomology, U. S. Department of Agriculture; and to Dr. E. C. Stakman of the Division of Plants Pathology and Botany, University of Minnesota, for helpful suggestions during the course of the study.

the possible rôle of these staining fungi in the death of the trees. In view of the great loss of timber attacked by beetles and bluestain fungi, an investigation of the fungi seemed advisable. Such was the purpose of the studies presented in this paper, based on work carried on at Asheville, N. C.

Historical review.

Von Schrenk (10)[5]) in 1903 published the results of his investigations of the death of large numbers of western yellow pines in the Black Hills Forest Reserve in South Dakota. He observed that several weeks after the trees were attacked by the Black Hills beetle (*Dendroctonus ponderosae* Hopk.) the wood became bluestained, and in the spring of the following year the foliage began to fade. However, he made no statements regarding the probable influence of bluestain on the death of the trees.

The result of Münch's (5) investigation appeared in 1907. His studies on the taxonomy and biology of bluestaining fungi contributed much to the knowledge of these organisms. Blocks of pine wood containing different amounts of water were inoculated with several species of Ceratostomella. The rate of fungus growth was found to be influenced by the amount of water and the corresponding amount of oxygen in the wood. Münch also inoculated living trees with species of Ceratostomella and obtained stain. Further observations led him to conclude that bluestain fungi were able to parasitize living parenchyma cells in the wood, provided the oxygen content of the wood was sufficiently high.

The most thorough investigation of bluestaining was made by Lagerberg, Lundberg, and Melin (4) in Sweden. They studied the important fungi which cause stain and described a number of new species. Their investigations on the effect of water and oxygen on the growth of bluestain fungi in pine blocks in general corroborated the results obtained by Münch.

Craighead (1) commented on the interrelation of bark beetles and bluestain, and Nelson and Beal (6) reported the results of a preliminary series of inoculations of bluestain fungi in living southern pines.

More recently, Grosmann (2) investigated the possibility of a symbiotic relation between bark beetles and fungi, and Rumbold (8) contributed further to the taxonomy of bluestain fungi associated with different species of bark beetles.

Bark beetles and their relation to bluestain.

Bark beetles of the genus Dendroctonus are said to be one of the most destructive groups of insects in the pine forests of the United States. The southern pine beetle

[5]) Reference is made by number (italic) to Literature Cited, p. 126.

(Dendroctonus frontalis), ordinarily endemic, under particularly favorable conditions becomes epidemic and causes widespread infestation in the southern forests. Pine which died after attack by *D. frontalis* during the past forty years is estimated by entomologists (9) to have been worth fifty million dollars. The life histories and habits of the southern pine beetle and associated beetles have been recorded in detail by Hopkins (3) and St. George and Beal (9).

The southern pine beetle attacks trees gregariously, the region of heaviest infestation being usually from 2 feet above the ground upwards 20 to 30 feet. It bores through the bark and excavates a winding S-shaped tunnel in the cambium region. Most of the tunnel is in the phloem, but the xylem is slightly marked. Eggs are deposited in niches along the tunnels and under favorable conditions a beetle may develop from egg to adult in about 40 days. Occasional trees may exude such a copious amount of resin during the boring of the entrance tunnels that the beetles are "pitched out". If infestation is successful and the insects become established, the color of the foliage of attacked trees begins to change after a few weeks. With the progressive loss of water, the foliage changes to a sorrel and then brown color, and death ensues.

Another group of bark beetles, commonly known as the engraver beetles *(Ips calligraphus* Germ., *I. grandicollis* Eich., and *I. avulsus* Eich.) are very commonly associated with the southern pine beetle. Under certain conditions the engraver beetles may become sufficiently numerous to attack vigorously growing pines, but they are, as a rule, of a secondary type and attack weakened trees.

A number of other species of bark beetles are found in dying pines. They are, however, of a secondary nature, and since they have not been studied in connection with this problem, no discussion of them will be included.

So far as is known, bluestain appears in the sapwood of all trees which have been successfully attacked by bark beetles. Occasionally some trees may be so resistant to insect attack and produce such a copious amount of resin that the beetles are not able to complete their life cycle. In such trees it is possible that bluestain fungi are also unable to thrive. Entomologists who have investigated the southern pine beetle problem are of the opinion that certain conditions unfavorable for beetle brood development are also unfavorable for the development of bluestain fungi.

Bluestain in living trees not attacked by bark beetles is apparently rare. The writer, in connection with these and other studies, has examined several thousand pines and in no case has he found in the trunk bluestain which could not be related to beetle attack. Stain not associated with insects has been observed in dry faces of turpentined trees; and Lagerberg, Lundberg, and Melin (4) have found stain in blazed spruces in Sweden. It appears that stain may be found in trees which have been severely wounded, but in such cases it is likely to be in relatively small amounts. Ordinarily in trees attacked by beetles in the South a considerable part of the sapwood becomes blued.

Observations and experiments reported in this paper were confined to three species of pine: shortleaf pine *(Pinus echinata* Mill.), pitch pine *(Pinus rigida* Mill.), and Virginia pine *(Pinus virginiana* Mill.).

Bluestain appears in the vicinity of the tunnels within a week or less after trees become infested with the southern pine beetle. The areas of infection vary greatly in size and shape, depending upon age; but as a rule their length considerably exceeds the width. They frequently coalesce to form patches of considerable size. When the bark is removed from the bluestained trees the exposed patches at the surface of the wood are black and bear numerous small sclerotia. On the cross-section of an infected stem areas appear as irregularly wedge-shaped sectors extending from the periphery to the heartwood (Fig. 1). The shape of the sectors is due to the hyphae growing from the outside towards the center along the medullary rays.

The method by which bluestain fungi gain entrance into pines has long been a subject for speculation. Most investigators of the subject have suggested that bark beetles probably carry the inoculum into the trees, but the opinion has also been advanced that the spores are blown or washed into the beetle tunnels.

Observations made by the writer during the course of the study led to some definite conclusions on this point. The inoculum can gain entrance only in two ways: through the entrance tunnels, or through ventilation tunnels made by the beetles at intervals along the completed galleries. Of 218 stained areas examined in shortleaf pines attacked by *Den-*

Figure 1.
Notch in stem of dead shortleaf pine showing stained sections caused by *Ceratostomella pini*.

droctonus frontalis, 97 per cent originated from entrance tunnels. Because of intermingling of galleries, the point of origin of the remaining 3 per cent could not be determined, but it is probable that they also came from entrance tunnels. If the inoculum were blown or washed into tunnels, areas of infection should have been found to originate from ventilation tunnels as well as from entrance tunnels.

Bluestain fungi were repeatedly isolated from bark beetles placed in agar. Although the majority of the bluestain cultures were obtained from insects taken from galleries which were stained, a number of them were also obtained from beetles taken from newly-made and unstained galleries. This evidence, although circumstantial, suggested that beetles carried bluestain inoculum on or within their bodies.

Additional evidence was provided by the phenomenon of specificity. Rumbold (8) made a detailed study of the bluestain fungi associated with bark beetles and reported

that *Ceratostomella ips* Rumbold was found only in association with species of Ips, and the vigorously growing strains of *C. pini* only in association with species of Dendroctonus. The writer made a large number of isolations from both beetles and blued wood and found the same close specific association.

The theory that beetles carry bluestain fungi into trees is strongly supported by the evidence obtained through the examination of entrance tunnels, from cultures obtained from beetles taken from unstained galleries, and by the phenomenon of specificity found independently by Rumbold and the writer.

From the studies here reported it is possible only to speculate regarding the nature of the inoculum and the method by which the beetles carry it into the tunnels. More information on the food requirements and life habits of the beetles is necessary before the details of inoculation can be learned. During the maturation of the beetle from larva to adult, it comes in contact with fungus mycelium and conidia, and when the adult emerges it undoubtedly carries some inoculum on its body. While boring the tunnel it would seem that the resin might effectively seal the inoculum to the body of the insect and prevent inoculation. It is therefore possibly more reasonable to assume that the inoculum is carried internally. The probable place of inoculation in a gallery, as indicated by the first appearance of the stain, is generally about one-half inch inward from the point of entrance of the beetle. If the inoculum were carried externally it would seem that the place of inoculation should be immediately at or near the entrance, although in some cases an extremely heavy flow of resin might inhibit hyphal growth. It is possible that the beetle, after overcoming the copious exudation of resin and excavating a short tunnel, makes an evacuation, and thereby inoculates the tree.

Ceratostomella pini and *Ceratostomella ips* in culture.

During the course of the investigation approximately 125 isolations of bluestain fungi were made from trees attacked by bark beetles, and approximately 175 beetles were plated on agar. Cultures were easily obtained by placing a small sliver from the interior of a piece of discolored wood on nutrient agar. Wood stained for a long time frequently produced molds and bacteria when cultured. When beetles were plated on Difco malt extract agar, yeasts and bacteria usually appeared first; but after some days the agar became slightly discolored beneath the beetle and gradually the bluestain organisms outgrew the others and covered the surface of the agar. In many cases the ends of the hyphae could be removed with a sterile needle and transferred to fresh agar without contamination. Molds and other fungi occasionally grew from the beetles so rapidly that no stain organisms could be obtained.

The writer, in 1928, made isolations from bluestained wood taken from trees attacked by various bark beetles; and, as has been previously stated, found a particular type of fungus associated with the southern pine beetle, another type associated with the engraver beetles, and still a third type with the turpentine beetles. Rumbold (7) had previously found that the fungus associated with the southern pine beetle was different from that associated with the engraver beetles, and in a later paper (8) described the two fungi in detail. She reported *Ceratostomella pini* associated with *Dendroctonus frontalis*, and a new species, *Ceratostomella ips*, with *Ips calligraphus* and *Ips grandicollis*. Although the writer did not make a comprehensive study of the taxonomy of these fungi, a brief description of the strains used in inoculation experiments is presented.

Mycelium of *Ceratostomella pini* grown on 2 per cent Difco malt extract agar, at room temperature, was at first scanty and colorless but within a week or less the agar became slightly darkened below the hyphal growth. The mycelium in older cultures formed a heavy, compacted, grayish mat, composed of large pigmented hyphae and narrow hyaline hyphae. Conidiophores bearing clusters of hyaline conidia, and perithecia, were produced in

this mycelial mat. Perithecia were also produced in abundance on the culture tube wall at the margin of hyphal growth, and were found submerged in the agar. The agar, at first amber colored, became slate gray in older cultures because of the gradual change in the color of the hyphae growing in the agar. Sclerotia and perithecia were produced on the agar surface as it dried and shrank from the wall of the culture tube. The perithecia of *C. pini* were extremely small. The basal part was almost spherical, and the neck was short and comparatively thick. The perithecia measured by the writer varied considerably but fell within the limits given by Rumbold (8). The sclerotia were black in mass and not uniform in outline and when crushed appeared to be constituted of brownish cellular plates, with thickened walls.

In young cultures of *Cerastomella ips* the aerial growth was relatively scanty except at the upper edge of the agar slant. Here the almost pure white, fluffy growth bore conidiophores in profusion. Such a fringe of white mycelium was not found in pure cultures of *C. pini* and was a good differentiating characteristic. Very thin, delicate, lacy, hyaline hyphae grew outward from the agar over the surface of the glass. These hyphae later became brown and pigmented and studded with perithecia. The surface of the agar in old cultures sometimes was covered with a thin, whitish, appressed hyphal growth which, however, never resembled the thick, grayish mat produced by *C. pini*. The perithecia of *C. ips* were much larger than those of *C. pini* and could easily be observed with the naked eye. They occurred closely grouped anywhere on the surface, solitary on the walls of the tube, or, very commonly, submerged in the agar. Sclerotia did not occur, as they did in *C. pini*.

Ceratostomella pini and *Ceratostomella ips* in sapwood of pines.

Although the hyphae of the bluestaining organisms were brown, the stained wood itself appeared bluish to the eye; a phenomenon discussed in some detail by Lagerberg, Lundberg, and Melin (4).

The writer observed that hyphae in wood blued by *Ceratostomella pini* were confined largely to the ray parenchyma cells and the resin ducts. In heavily stained wood, practically all of the ray parenchyma cells were filled with large, brown, septate hyphae. Strands of hyphae extended from these cells into the wood tracheids and sometimes grew for long distances. Usually not more than a single hypha was found in the lumen of a wood tracheid, and hyphae in the ray tracheids were rarely found. Their method of penetration from one cell to another was ordinarily through the pits, but occasionally a single thread penetrated through the walls of 7 or 8 tracheids. The hyphae, when they penetrated directly, were very greatly constricted and sometimes formed appresoria. Apparently these fungi were usually unable to dissolve the thick tracheid walls but were able to break down the end walls of the ray parenchyma cells.

The cell walls in wood stained by *Ceratostomella ips* appeared to be only slightly discolored. Hyphae were concentrated in the ray parenchyma as in *C. pini*, and hour-glass constrictions decribed by Lagerberg, Lundberg, and Melin (4) were observed occasionally in rays two cells high. In some parenchyma cells the hyphae developed so as to suggest a grouping around the nucleus, and coils of hyphae were occasionally found in the hollows of bordered pits.

The bluestain fungi grew at a rapid rate radially through the medullary rays and vertically through the lumina of the tracheids and through the resin ducts. As was to be expected, growth in a tangential direction was very much slower. The most rapid growth observed was in a shortleaf pine inoculated with *Ceratostomella pini*. In 6 days the stain had penetrated radially an inch (about 7 annual rings) and had spread above the point of inoculation about 3 inches.

Inoculation of pines with *Ceratostomella pini* and *Ceratostomella ips.*

Inoculations of living pines with bluestain fungi were first reported by Münch (5). He believed that to be susceptible to bluing, the sapwood at the place of inoculation must have a higher oxygen content than is found in wood with the ordinary water content. For this reason he treated a number of pines and spruces in different ways to decrease their moisture content before inoculating them with Ceratostomella. Results were negative except in the case of two pines which had been inoculated between two saw cuts one-half meter apart. Münch concluded that under certain circumstances bluestain fungi could kill living trees.

Lagerberg, Lundberg, and Melin (4), in discussing the work of other observers, stated that bluestain fungi conceivably contributed to the death of trees ordinarily considered to be killed entirely by the attack of pine beetles.

Craighead (1) and Nelson and Beal (6) believed that bluestain was an important factor in the death of trees attacked by the southern pine beetle. The belief of Nelson and Beal was supported by a series of inoculation experiments made in 1928. The detailed results have been published (6), but for the sake of continuity they will be briefly summarized here.

Three types of Ceratostomella *(C. pini, C. ips,* and *C. rom.)* were isolated from shortleaf pine attacked by *Dendroctonus frontalis, Ips calligraphus,* and *Dendroctonus terebrans,* respectively, and grown on boiled and autoclaved rice or on malt extract agar. Shortleaf pines *(Pinus echinata* Mill.) and pitch pines *(Pinus rigida* Mill.) 4 to 8 inches in diameter at breast height were inoculated by three methods: (1) poultice, (2) through drill holes, and (3) with a grease gun. Rice was the substratum in methods 1 and 3 and malt agar in method 2. By the end of 4 months, all trees were stained and 15 of the 19 trees in Series 1 and 3 were dead or dying. The 3 trees inoculated through drill holes were less severely stained and remained alive until they were cut, a year after inoculation. The 4 check trees examined 18 months after treatment were alive and normal in appearance.

1929 and 1930 experiments.

Series A.

In the summer of 1929 additional inoculation experiments were made. Twelve shortleaf pines ranging from 20 to 35 feet in height and from $3^1/_2$ to $4^1/_2$ inches in diameter at breast height were inoculated through nail holes with *Ceratostomella ips.* Before inoculation, spike holes were punched through the bark and a short distance into the xylem. The holes were one inch apart and extended in circles around the trees at various

heights above the ground. In each hole was placed approximately 3 drops of inoculum, which was made by macerating in tap water the mycelium and perithecia scraped from the surface of malt agar cultures of *C. ips*. Twenty-seven months after inoculation none of the trees were infected with the bluestain organism, and all except one were living. The reasons for the results of this experiment are not known but it is possible that an excessively high water content in the trees and a considerable exudation of pitch inhibited fungus growth.

Series B.

Several shortleaf pines were also inoculated with strips of inner bark from pines infected with *Ceratostomella pini*. No stain resulted from these inoculations and all trees were alive at the end of 27 months.

Series C.

Thirteen pitch pines were inoculated in girdles with *Ceratostomella pini*. The trees were of the same size as those in the previous series. Before inoculation 3 of the trees were girdled by a 2-inch strip 2 feet above ground, 3 at midstem, and 3 at the base of the crown; and the remaining 4 trees were girdled by 3 strips, 2 feet above ground, midstem, and base of crown. Malt agar cultures of *C. pini* were smeared on the surface of the wood exposed by the girdles and the bark was refastened with nails. Girdles on 5 check trees were smeared with sterile agar.

Six months after inoculation 2 of the 4 trees inoculated in 3 places had died. In the boles of both of these, sections 2 feet or more long were completely stained. Eight months after inoculation all the other trees in the series were alive, but 27 months after inoculation all except one check tree had died. None of these showed any evidence of bluestain resulting from inoculation, but had died from the effects of girdling and the action of secondary bark beetles. Apparently the malt agar had dried rather quickly and did not provide a favorable substrate for the growth of *C. pini* in the girdles.

Series D.

In August, three 3 8-inch to 2-inch branches on each of 6 Virginia pines *(Pinus virginiana* Mill.) were inoculated with poultices of *Ceratostomella ips*. An inch girdle through the phloem was made at 3 to 4 feet from the trunk, a poultice of *C. ips* on rice was applied on each branch, and the bark was fastened with tacks. One check branch on each tree was poulticed in a similar manner with sterile rice.

About 6 months after inoculation, 4 inoculated branches were dead. Of these, one was heavily stained and the other 3 were slightly stained in the 2 or 3 outer rings. The foliage on the remaining 14 inoculated

branches and of the 6 check branches was alive, and no stain could be found in the poulticed areas.

Series E.

Twelve shortleaf pines, 25 to 30 feet in height and 2 to 3 inches in diameter at breast height, were inoculated with a spore suspension of *Ceratostomella pini* obtained by macerating in tap water the mycelial mat of the fungus grown on malt agar. The outer flaky bark of the trees was first removed with a draw shave for a distance of approximately 10 feet, beginning at 2 feet above the ground. Great care was exercised not to injure the living inner tissues. A short tunnel was made with a curved bag needle inserted between the phloem and xylem for a distance of approximately one inch at an angle of 45°. The hypodermic needle was then introduced into the tunnel and the inoculum gently injected. Usually not less than one-half c. c. nor more than 1 c. c. was injected at each place. The 50 to 70 points of inoculation were spaced fairly evenly over the surface of an 8- to 10-foot length of bole, being 5 to 6 inches apart vertically and 2 to 4 inches apart obliquely. Four check trees of the same size were similarly treated, except that tap water instead of a spore suspension was injected.

Eight months after inoculation many of the inoculated trees in this series were stained but only one was dead. The volume of sapwood stained varied greatly; in one tree only a few small areas of infection were observed, whereas in another, three-fourths of the cross-section of stem for a short vertical distance was discolored. The tree that died during the course of the study succumbed within 6 months after inoculation, and the sapwood between 4 and 6 feet above the ground was seven-eighths blued. It is probable that some of the other more severely stained individuals might have succumbed had they not been cut and sectioned for moisture content determinations. Two years after treatment 7 inoculated and 3 check trees that remained were cut and sectioned. The foliage on all was green and apparently healthy. Of the 12 trees inoculated in this series, 8 became stained.

Series F.

In September, 12 shortleaf pines were inoculated by the grease-gun method with cultures of bluestain fungi grown on rice. The trees were of the same size as those in Series E and were given the same treatment preparatory to inoculation. The rice fungus mixture was stirred with water to a semi-liquid consistency and was injected with a grease gun into tunnels made with a curved bag needle. From 50 to 75 inoculations were made on each tree over an 8- to 10-foot length of bole. Six trees were inoculated with *Ceratostomella pini*, 6 with *C. ips*, and 4 check trees were injected with sterile rice.

After an interval of 2½ months, 3 of the 6 trees inoculated with *Ceratostomella pini* were dead and one was dying. These trees, when examined, were severely stained, a longitudinal section of the sapwood 2 to 4 feet in length being completely blued. Other trees in this series, with apparently healthy foliage, also were found to be heavily stained. In such trees, however, no cross-sections were completely discolored. Presumably the transpiration stream was capable of passing around stained areas and if there was between the roots and the crown a channel, even though circuitous, which was not blocked by bluestain, the tree remained alive. In some trees as much as 80 per cent of the cross-section of the stem at certain heights had been blued and yet the tree was able to transport sufficient water to keep its foliage alive. Eleven of the 12 trees inoculated in this series became stained. The foliage of the 4 check trees remained healthy throughout the course of the experiment.

Series G.

In June, 1930, an additional group of 9 shortleaf pine trees, ranging in height from 13 to 22 feet, and in diameter from 1½ to 3 inches breast high, were inoculated by the poultice method. The inoculum consisted of *Ceratostomella pini* grown on rice, as in other series, but in addition it contained a large amount of bacterial material. The unidentified bacterium present was isolated with the fungus from blued wood of a tree attacked by *Dendroctonus frontalis*. A 4-inch girdle of bark was removed from each tree at breast height, a poultice of the organism and rice applied to the surface of the wood, and the bark replaced and fastened. Three check trees were similarly girdled but were treated with sterile rice.

Within 4 weeks after inoculation, one tree was dead and 2 others were dying; within 5 weeks, 3 more were dying; and after 10 weeks, 2 of the remaining 3 inoculated trees were dying. Because individuals were sometimes cut and sectioned shortly after the foliage was beginning to change in color the probable time that death would have ensued is not known.

Unfortunately, as most of the trees after inoculation were soon attacked by Ips bark beetles, results from this series are less conclusive than those from the other series.

Check trees when cut were in a healthy condition.

Discussion of inoculation results.

Infection after inoculation and the rapidity with which the infection spread in a tree was dependent upon some factors yet unknown. Possibly the most important one was the water content of the tree. Seasonal and yearly fluctuations, as well as differences in individual trees, no doubt

influenced the rapidity of growth of the organisms. More difficulty was encountered in infecting trees during the summer of 1929 than in the summers of 1928 and 1930, but insufficient information is available to permit more than a conjecture as to the reason.

Another factor which influenced the degree of infection after inoculation was the type of inoculum used. The best results were obtained by using boiled rice as a substrate for carrying the inoculum. Results obtained in 1929 indicated that agar as a medium dried rapidly and the fungus often failed to become established. However, one series of trees was inoculated with a spore suspension with fairly good results.

The degree of wounding which accompanies the inoculation of a tree may conceivably influence the rapidity of the infection, if the wounds are very large. In the data so far presented, however, there is no evidence that the check trees were severely injured, except in those cases where treatment consisted of complete girdling one or more times, or when the trees were attacked by bark beetles after treatment. In many cases, particularly when they were treated with tap water by means of a hypodermic needle, the wounds were partly or completely callused over. With exception of those specifically mentioned, all check trees remained alive until cut.

Excluding Series G because of insect infestation and Series D because it consisted of branch inoculations and is not comparable with the other series, a total of 74 trees were inoculated. Of this number, the death of 22 trees was attributed to the action of bluestain fungi. It should be pointed out, however, that in no case were the inoculations comparable in type or degree with inoculation of trees by bark beetles. It is impossible to simulate either insect tunnels or the type of inoculum carried by insects.

Inoculation experiments with the bluestain fungi, *Ceratostomella pini* and *C. ips*, in pines demonstrate that these fungi will grow readily, and sometimes with considerable rapidity, in the sapwood of living pines. It appears that a considerable volume of the sapwood of a tree may be stained and the tree may still remain alive. However, if the entire cross-section of the trunk becomes stained, death invariably ensues.

Experiments on water relations of pines.

Numerous investigators have observed that pines attacked by bark beetles and bluestain fungi appeared to dry out rapidly and that sectors of blued wood in such trees could frequently be determined by inspection to be much drier than adjacent unstained sectors. The unpublished moisture determinations by Craighead, St. George, and Beal, previous to the beginning of the investigations reported in this paper, demonstrated great differences between trees attacked by the southern pine beetle and unattacked trees.

It appeared that a comparison of the water content of trees attacked by beetles, trees inoculated with bluestain fungi, and check trees, would shed further light on the effect of the fungi, and a series of measurements were therefore made.

Table 1.

Description of *Pinus echinata* trees at time of cutting for moisture determinations in 1929.

Tree No.	Height Feet	Diameter breast high Inches	Age	Condition	Date of bark beetle attack	Severity of bark beetle attack	Date of topping	Interval after topping Days	Interval after attack Days
a	35	4.5	20	Normal	—	—	—	—	—
b	43	7.7	24	Normal	—	—	—	—	—
c	30	5 0	20	Topped	—	—	August 3	13	—
d	30	4.6	18	Topped	—		August 3	13	—
e	29	4.2	23	Attacked	July 20	Medium	—	—	27
f	32	4.5	22	Attacked	July 5	Fairly heavy	—	—	42
g	30	6.9	21	Attacked and topped	July 20	Fairly heavy	July 31	16	27
h	32	6.9	22	Attacked and topped	July 10	Fairly heavy	July 31	16	37

Comparison of unattacked pines with those infested with *Dendroctonus frontalis* [6]).

During the summer of 1929, moisture determinations were made of pines attacked by *Dendroctonus frontalis* and of unattacked trees. Table 1 gives a partial description of 8 shortleaf pines and the method by which they were treated. Discs one-half to three-fourths inch thick were taken at intervals of 2 feet, beginning at 2 feet above the ground and extending upward to the base of the crown. The bark and phloem were immediately removed from the discs, and the remainder weighed. The discs were then dried for 46 hours at temperatures between 90° and 94° C, and the moisture content computed on a dry-weight basis.

Graphs of the water content of these trees are presented in Figure 2. It appears that, for the trees examined:

(1) The water content in normal trees was lowest at the base and increased with an increase in height.

(2) Topping normal trees caused an accumulation of water within the tree but otherwise the trend was the same as in untopped trees.

(3) The moisture content of trees attacked by the southern pine beetle was highest at the base, and decreased with an increase in height, being the reverse of the condition found in normal trees.

[6]) Cooperative experiment with R. A. St. George of the Division of Forest Insects, Bureau of Entomology, U. S. Department of Agriculture.

(4) In trees attacked by bark beetles but decapitated, moisture contents at different heights were variable, depending probably on the period of beetle infestation, time and period of decapitation, and amount and distribution of bluestain within the trunk.

Additional data (Tables 2 and 3) on the moisture content of shortleaf pines attacked by the southern pine beetle were obtained during the

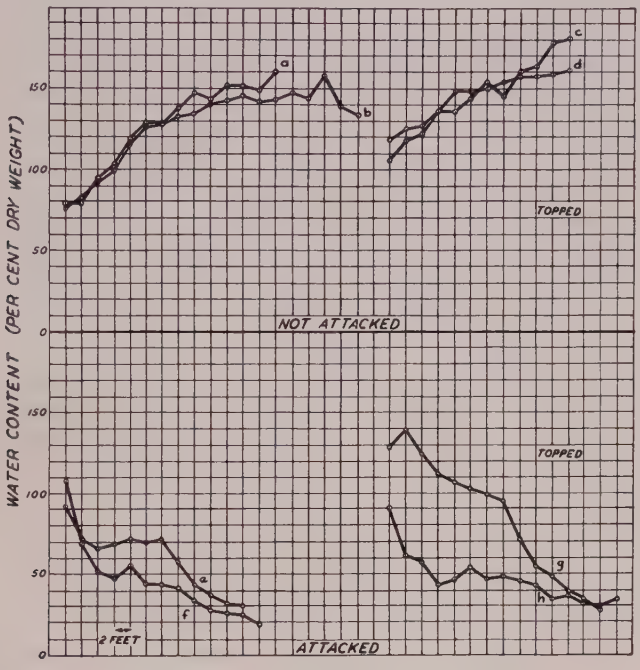


Figure 2.
Water content at 2-foot intervals in shortleaf pines.
Trees a and b not attacked and not topped; c and d not attacked but topped;
e and f attacked by *Dendroctonus frontalis* but not topped;
g and h attacked by *Dendroctonus frontalis* and topped.

summer of 1929. Sections were taken at every 2 feet beginning at 2 feet above ground, and moisture determinations made in a manner previously described. A brief description of the trees will be found in Table 2. Because the degree of attack, amount of bluestained wood, length of attack, and difference in height and diameter of individual trees were variables, it was to be expected that the moisture contents, at a given height, were not the same for all trees. The curves of moisture content for these trees are fairly similar, being high at the base and decreasing with the height.

The lowered water content of attacked trees can be attributed chiefly to one of two causes. The water is lost either through the tunnels

Table 2.

Description of *Pinus echinata* trees, attacked by *Dendroctonus frontalis*, at time of cutting for moisture determination in 1929.

Tree No.	Height Feet	Diameter breast high Inches	Date of attack	Date cut	Stage of beetle brood development	Condition of foliage
54	30	5.5	July 13	August 21	Mature larvae and pupae	Turning brown
57	28	5.4	July 13	August 12	Mature larvae	1/2 top brown
26	35	6.1	July 6	August 12	Mature adults	Top dead
59	32	5.8	July 6	August 12	Mature adults	Top dead
29	38	5.4	July 5	August 12	Pupae and mature adults	Top dead
44	40	10.0	July 5	August 13	Pupae and mature adults	Top dead

Table 3.

Percentage of moisture based on oven-dry weight in sections cut from 6 *Pinus echinata* trees attacked by *Dendroctonus frontalis*.

Height of section Feet	Percentage of moisture in trees of indicated numbers					
	54	57	26	59	29	44
2	90	100	97	90	100	119
4	62	68	65	64	64	132
6	67	66	58	48	69	113
8	68	63	62	52	83	104
10	74	56	62	50	73	96
12	71	56	50	58	70	95
14	64	37	58	51	70	90
16	43	36	47	47	73	72
18	38	41	51	43	69	68
20	39	31	40	40	63	67
22	—	—	43	46	57	67
24	—	—	46	52	50	56
26	—	—	48	51	45	56
28	—	—	66	—	42	39
30	—	—	52	—	34	35
32	—	—	—	—	—	41
34	—	—	—	—	—	41
36	—	—	—	—	—	50

of the beetles or it is prevented from passing upwards into the tree by the blued zones in the sapwood in the lower part of the bole. Since it is impossible to simulate beetle tunnels experimentally, the degree to which water is lost through such wounds is not known. Evidence, however, on the effect of blued wood on water conduction is provided by the following moisture determinations on inoculated and check trees.

Comparison of uninoculated trees with those inoculated with Ceratostomella.

A number of inoculated trees from Series E and F were cut and sectioned in April, 1930. Discs were taken at 2-foot intervals, beginning at

2 feet above ground and extending upward to the base of the crown, and their moisture content determined on a dry-weight basis. Results are presented in Figure 3, A. The water content of the untreated tree, a,

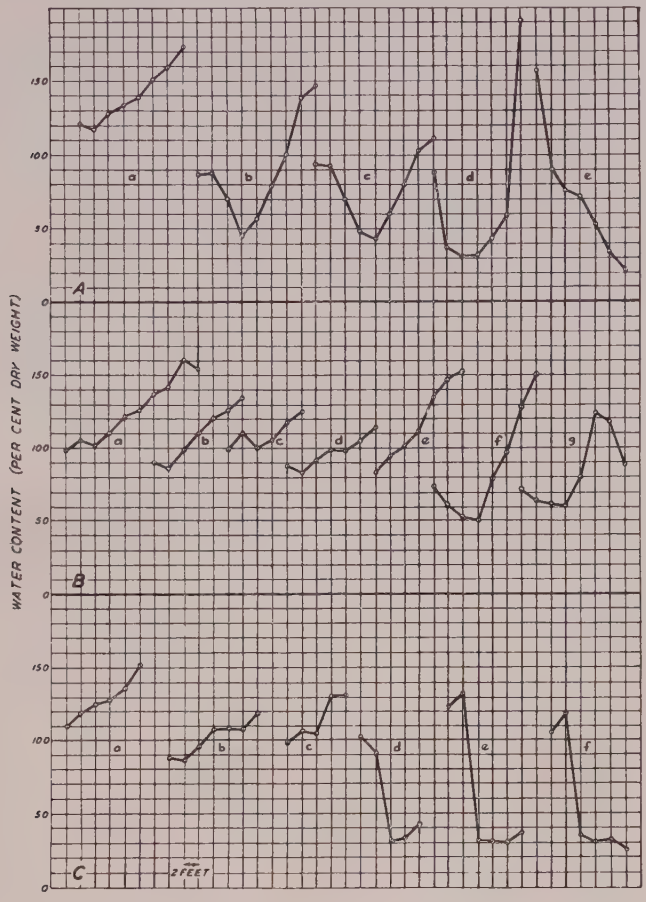


Figure 3.

Water content at 2-foot intervals of inoculated and check shortleaf pines. A, Trees cut on April 1, 1930: a, untreated tree; b and c, inoculated, severely stained, foliage green; d, inoculated, very severely stained, foliage turning brown; e, inoculated, bole between 5- and 10-foot sections completely stained, tree dead for several months. B, Trees cut on June 24, 1930: a and b, untreated trees; c and d, check trees, unstained, foliage green; e, inoculated, unstained, foliage green; f and g, inoculated, sapwood approximately half stained, foliage green. C, Trees cut July 29, 1930: a, untreated tree; b, check tree, unstained, foliage green; c, inoculated, unstained, foliage green; d and e, inoculated, very severely stained, foliage turning brown; f, inoculated, very severely stained, foliage dead.

differed decidedly from that of the other 4 trees which were inoculated and had become heavily stained. Trees b, c, and d, inoculated in the region between 2 and 10 feet, were severely stained but still alive. In these trees

the water content decreased from the base toward the central or upper part of the stained region, and from then upward, increased with an increase in height of the tree. Tree e, dead, and more severely stained than the others, had a moisture curve almost the reverse of the untreated tree.

A second group of trees from Series E und F were cut and sectioned approximately 3 months later. The trend of the curves a, b, f, and g, in Figure 3, B, are similar to curves for comparable trees in the first group. In addition, check tree c, treated with sterile rice, check tree d, treated with tap water, and inoculated but unstained tree e had water contents differing but slightly from those of untreated trees.

In Figure 3, C, are curves for trees taken from Series G. Trees a, b, and c present no outstanding differences from comparable trees in the other groups. Those represented by d, e, and f, however, deserve further consideration. The water content increased from the 2-foot to the 4-foot section in 2 of the 3, and in the third, decreased only slightly. Yet between the 4-foot and 6-foot sections, the region of inoculation and stain, there was a very great and sudden decrease.

The remaining 15 trees in Series E and F, together with 5 untreated trees, were cut in the fall of 1931. The trends of the curves for the various types corresponded very closely with those previously described and a discussion of this group is therefore omitted.

Results of the examination of 18 trees, presented in Figure 3, together with 15 others the data for which are not given, can be summarized as follows: In untreated green trees, in check trees treated with sterile rice or water, and in inoculated trees which remained unstained, the water content increased with the height. In partly stained trees, the water content decreased abruptly in the region of stain and increased above this region. In dead trees the percentage of moisture decreased with the height, the reverse of conditions found in green untreated trees. It should be pointed out that in the trees discussed practically the entire bole consisted of sapwood, the heartwood being restricted to a narrow cylinder in the center of the tree.

Several discs were cut at 2 feet above ground from one of the trees which had been inoculated with a grease gun. Approximately one-half of the sapwood in an 8-foot length of bole had become stained but the foliage was still green. The stain had spread from the points of inoculation in such a way that in any cross-section through the trunk 3 sectors of blued wood alternated with 3 sectors of clean wood. The average moisture content of the blue sectors was 28 per cent; whereas the moisture content of the clean wood was 94 per cent.

Evidence presented in the foregoing graphs and tables strongly suggests that bluestain in a tree interferes with the upward conduction of water, and that if a sufficient amount of the sapwood is blued death en-

sues. The fungus kills the phloem and cambium at the point of inoculation, causing wounds through which a certain amount of drying toward the outside probably takes place. But graphs of the check trees show that wounds produced mechanically by inoculation are of comparatively little importance in this respect. For these reasons, it is believed that the chief cause for the water reduction in both beetle-attacked and inoculated trees is the presence of bluestained areas and not evaporation through wounds made either by beetles or as a result of inoculation.

Experiments on ascent of dye in pine saplings.

Earlier investigations during the course of the study having indicated that blued areas caused a stoppage of the transpiration stream, additional experiments were made to obtain more detailed information regarding such stoppage. Pine saplings which could be manipulated easily were accordingly treated in several ways and set in dye solutions. In this way the path of the ascending water stream could presumably be traced by the dyed areas in the wood.

Comparison of living and killed saplings.

A very limited incidental experiment on the effect of killing solutions on the subsequent rise of dye in stems of shortleaf pine saplings was first made. Twelve saplings averaging 6 feet tall were cut off at the ground and the butts immediately dipped in melted paraffine. A 4- to 6-inch section was cut from the base of each while the base was submerged in water. Six saplings were then placed in mason jars containing a saturated solution of picric acid, and 6 others were placed in tap

Table 4.

Ascent of picric acid and dye in shortleaf pine saplings.

Tree	Height	Diameter	Crown condition	Amount of liquid absorbed			Height of ascent	
				Acid	Water	Dye	Acid	Dye
No.	Feet	Inches		ccm	ccm	ccm	Feet	Feet
In picric acid 22 hours — In Licht Grün 25 hours								
276	5.7	1.3	Heavy	150	—	24	$2^1/_2+$	1 +
277	6.3	1.4	Medium	160	—	40	3 +	1 +
278	6.0	1.4	Medium	30	—	20	2 +	$^1/_2$ +
279	6.9	1.5	Heavy	170	—	23	$2^1/_2+$	$^1/_2$ +
280	5.5	1.3	Medium	105	—	37	$3^1/_2+$	0 +
281	5.5	1.2	Medium	80	—	25	3 +	1 +
In tap water 22 hours — In Licht Grün 25 hours								
282	5.9	1.3	Medium	—	135	85	—	$1^1/_2+$
283	6.0	1.4	Medium	—	240	151	—	$3^1/_2+$
284	6.0	1.2	Medium	—	225	143	—	3 +
285	6.5	1.6	Heavy	—	370	169	—	3 +
286	5.4	1.1	Heavy	—	215	164	—	3 +
287	5.5	1.1	Medium	—	125	70	—	$1^1/_2+$

water. At the end of 22 hours all were transferred to jars containing one-tenth per cent solution of Licht Grün F. S. Grübler. After 25 hours the plants were removed and sectioned. A description of the plants, the height of ascent of liquids, and the amount of liquids absorbed are give in Table 4.

The dye ascended decidedly higher in check saplings than in saplings set in picric acid in which the living cells had been killed. These results are at variance with those obtained by investigators who have used similar experimental methods in an attempt to prove that living cells are not necessary to the upward conduction of water in plants.

Comparison of uninoculated saplings with those inoculated with Ceratostomella.

Twelve saplings were inoculated with rice-bluestain poultice of *Ceratostomella ips*, 12 were treated with sterile rice, and 12 were left untreated. The fungus used for inoculum was obtained from blued wood of a shortleaf pine attacked by *Ips grandicollis*. A 3-inch wide girdle of bark was removed from the saplings at a point 2 feet above the ground, a poultice of rice bluestain or sterile rice applied, and the bark replaced and tied with cord. A month after inoculation the first series, consisting of 6 individuals from each of the 3 above-mentioned groups, was removed for test. The saplings were cut off at the ground and the butts immediately dipped in melted paraffine. The basal portions were submerged in water and a 12- to 18-inch section removed with a hack saw. Each tree was then quickly lifted from the water and transferred to a pint jar about half full of a one-fourth of one per cent solution of Grübler's Licht Grün F. S. These (Series A) plants were removed from the dye and examined approximately 20 hours later. Three days later, 18 other saplings (Series B) were set up in the manner just described. Some of the plants in this experiment were removed from the dye after 27 hours; the remainder were left for 50 hours. During this period a rain occurred which lasted continuously for 36 hours.

The results of both experiments are recorded in Table 5. In Series A and B the dye passed rapidly upward without exception in the uninoculated saplings, ascending almost as high in saplings poulticed with rice, with the exception of numbers 132, 133, and 134. In these individuals the reason for stoppage is not definitely known. Sections were cut from the poulticed area and a large number of hyaline hyphae were observed to extend chiefly through the tracheids. Hyphae could also be observed occasionally within the ray parenchyma cells, but the protoplasmic contents of these cells were so deeply stained that hyphae may have been present in greater numbers than was observed. Dye ascended only a few inches in 7 of 12 saplings inoculated with bluestain. Of the remaining 5, number 152 is taken as an example and the path of the dye solution which

ascended 30 inches is illustrated in Figure 4. It will be observed that there was a gradual and progressive increase in area of stoppage from the base to the middle of the inoculated region. Above the midpoint of the poulticed region, the area of stoppage decreased. It is evident that as

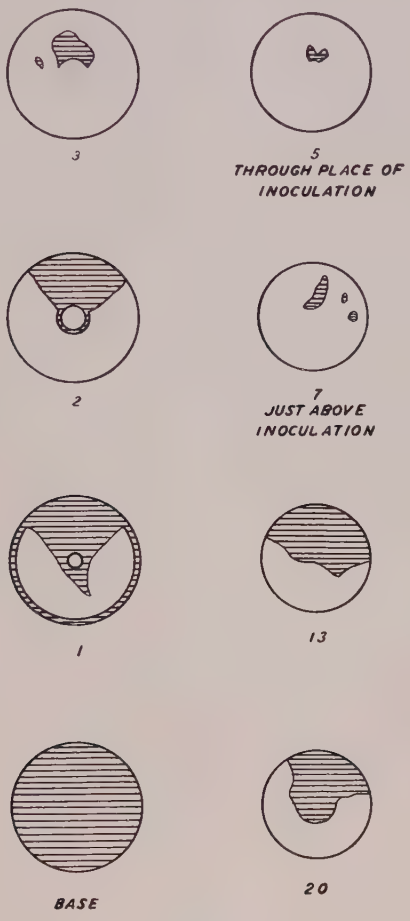


Figure 4.

Oriented diagrams of cross section from shortleaf pine sapling No. 152 inoculated with *Ceratostomella ips*. Lined areas represent the part of stem which was dyed. Figures beneath diagrams indicate height of section in inches above base.

the solution passed from the base upward, the stream was forced inward and restricted to a very small passage through the inoculated area, after which the stream expanded and continued upward in a more or less normal manner.

The solution ascended higher in the untreated trees in Series B than in Series A. The trees were somewhat larger and were allowed to stand longer in the solution. Inoculated trees in the second series had consid-

Table 5.

Effect of inoculation with *Ceratostomella* sp. upon the ascent of dye in shortleaf pine saplings.

Uninoculated				Poulticed with sterile rice				Poulticed with rice bluestain			
Tree No.	Height Feet	Diameter Inches	Ascent of dye Inches	Tree No.	Height Feet	Diameter Inches	Ascent of dye Inches	Tree No.	Height Feet	Diameter Inches	Ascent of dye Inches
Series A [1]											
207	7.5	1.3	50	129	10.0	1.4	80	135	10.0	1.2	3
208	8.5	1.5	50	130	11.0	1.4	62	136	10.0	1.2	3
209	10.0	1.4	50	131	6.0	1.3	42	139	6.0	0.9	4
210	8.0	1.6	50	133	7.0	1.0	2	149	7.0	1.5	4
211	8.0	1.2	55	134	6.0	1.0	3	152	8.0	1.8	30
212	8.0	1.2	65	142	11.0	1.7	92	162	8.0	2.1	4
Series B [2]											
213	7.5	1.3	90	132	6.0	0.7	2	137 !	12.0	1.9	33
214	8.0	1.6	95	141	10.0	1.9	96	138 !	11.0	1.5	50
215	15.0	1.8	80	145 !	11.0	1.8	42	140 !	9.0	1.2	12
216	9.0	1.5	80	146 !	9.0	1.4	30	147 !	10.0	1.8	5
217	6.0	1.2	60	156 !	13.0	2.4	40	150 !	10.0	1.8	6
218	9.0	1.7	65	158 !	14.0	2.0	36	161 !	12.0	2.1	15

erably less blued wood than those in the first series, and it will be observed that the solution ascended considerably higher in the former.

The results of the foregoing experiments can be summarized as follows: Untreated trees placed in an aqueous solution of Licht Grün rapidly absorbed the solution, which ascended almost to the very tip in the course of a day. With a few exceptions, the ascent was almost as rapid in trees poulticed with sterile rice. In the trees poulticed with bluestain fungi, stoppage at the inoculated areas was complete in many cases, and in the remainder upward flow was restricted to small areas toward the center of the trees. The data here given indicated that bluestained areas in living saplings checked the upward flow of water.

Comparison of uninoculated saplings with those inoculated with Trichoderma.

A green mold, *Trichoderma* sp., is very commonly found in the tunnels of bark beetles, and, although it was believed to be merely saprophytic, a number of pine saplings were inoculated in order to determine whether the action of this fungus was similar to Ceratostomella. The organism was collected from a southern pine beetle tunnel and grown on rice. Eighteen shortleaf pine saplings were inoculated with a rice-Trichoderma mixture, and 18 were treated with a poultice of sterile rice. The experimental method was the same as that used in the foregoing experi-

[1]) Left in stain for 20 hours.

[2]) Left in stain for 27 hours except those marked (!) which were left for 50 hours.

Table 6.

Effect of inoculation with *Trichoderma* rom. upon the ascent of Licht Grün and Orange G in shortleaf pine saplings.

Untreated					Poulticed with sterile rice					Poulticed with Trichoderma rice				
			Ascent of dye					Ascent of dye					Ascent of dye	
Tree No.	Height	Diameter	Orange G	Licht Grün	Tree No.	Height	Diameter	Orange G	Licht Grün	Tree No.	Height	Diameter	Orange G	Licht Grün
	Ft.	In.	Ft.	Ft.		Ft.	In.	Ft.	Ft.		Ft.	In.	Ft.	Ft.
333	6.5	1.4	—	3½+	312	6.6	1.5	—	4+	288	6.7	1.3	—	5
334	6.8	1.5	—	3½+	316	7.0	1.2	—	5+	298	6.6	1.4	—	4
335	6.4	1.1	—	5+	318	6.4	1.4	—	3½+	302	6.6	1.8	—	3+
336	6.5	1.4	4½+	—	307	7.2	1.5	5½	—	290	7.4	1.6	6	—
337	6.6	1.2	4½+	—	310	7.1	1.4	4+	—	292	6.7	1.5	4½+	—
338	6.5	1.2	5+	—	315	7.0	1.1	6+	—	293	6.5	1.4	4½	—

ment. Fifteen days after inoculation 6 saplings from each of the 2 groups, together with 6 untreated individuals, were cut and placed in dye solutions. One-half of the total number were placed in a one-twelfth of one per cent solution of Licht Grün F. S. and the remainder in a one-tenth of one per cent of Orange G. Twenty to 22 hours later the saplings were removed and sectioned. The results of this experiment are given in Table 6. The average height of ascent of dye in saplings untreated, treated with sterile rice, and inoculated with Trichoderma was 4⅓, 4⅔, and 4½ feet, respectively. It is apparent that Trichoderma was not actively parasitic and when inoculated into saplings has no greater disturbing action than sterile rice.

The remaining 24 inoculated and check saplings were examined approximately 2½ months after treatment and the foliage of all except one appeared to be normal.

Comparison of tori in stained and unstained wood.

Results of the investigation reported so far indicated that bluestain in the sagwood of pines caused a stoppage of the transpiration current. The reason for stoppage, however, remained in doubt. An anatomical examination of sapwood from newly felled pines was therefore made, with the following results.

The water-conducting tissues in the xylem of a conifer consist of comparatively long, slender, thick-walled tracheids connected on their radial walls by bordered pits. The ascending transpiration current passes through the lumina of tracheids into contiguous tracheids above, through the perforations in the pit membranes. Suspended by the membrane in a bordered pit is the torus, a thickened plug which serves as a valve. If

the torus is in a median position, water is unimpeded in its progress through the bordered pit. If, however, the torus is aspirated, forced against either opening of the pit, water is unable to pass through the pit.

The degree of aspiration of tori in a section of wood appears to be correlated with the degree of permeability of the wood to liquids. Scarth[7]) believed that the death of living ray parenchyma cells in pines preceded or accompanied a change from permeability to impermeability because the contents of the cells or decomposition products resulting from the death of these cells appeared in the conducting tracts and occluded the openings in the pit membranes. The tension of the sap column then caused the tori to become deflected to the sides of the pits.

Table 7.

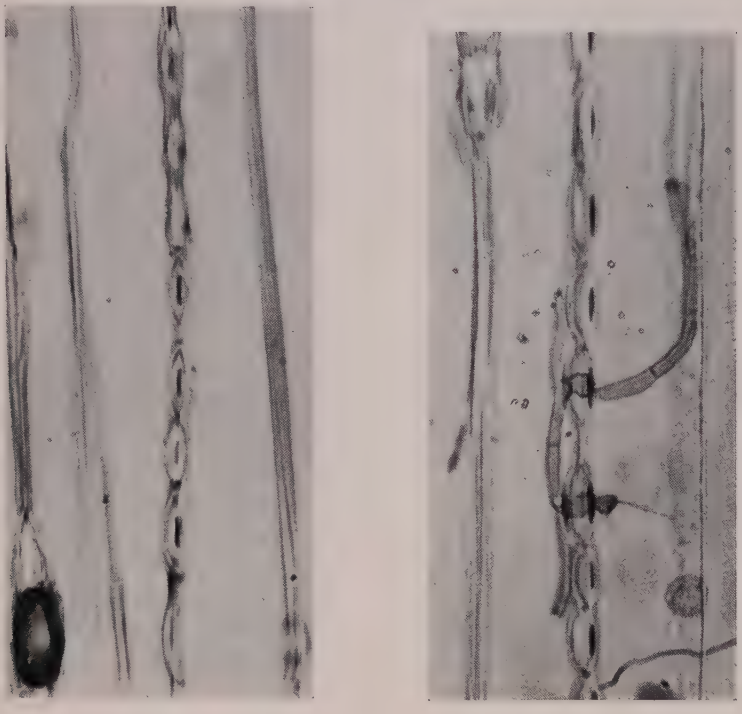
Percentage of aspirated tori in stained and unstained wood of shortleaf pines attacked by *Dendroctonus frontalis*.

Tree No.	Development of beetle brood	Type of material	Number of tori examined	Per cent of tori aspirated
1	Unattacked tree . . .	Green	1560	2
	Foliage green	Dried in laboratory 10 days	512	97
2	Adults extending tunnels. Foliage green . . .	No stain	1544	1
3	Adults and eggs . . .	No stain	1582	22
	Foliage green	Very slightly stained . . .	1798	24
4	Almost mature larvae .	No stain, wood moist . . .	1785	80
	Foliage green	No stain, wood dry . . .	1708	87
		Stained	1640	91
5	Callow adults	No stain	1747	90
	Foliage reddish brown .	Stained	1669	93

Since ray parenchyma cells in bluestained wood were unquestionably killed by the invading fungus, and such stained wood in living trees interfered with the passage of the transpiration current, pines with different degrees of insect development and fungus discoloration were examined (Table 7) for the purpose of determining the condition of the tori. In the unattacked tree and the tree so newly attacked that stain had not yet gained a foothold, the tori were almost entirely in a median position. (Fig. 5, A.) In tree No. 3 where the fungus had had a brief opportunity to invade the wood, a greater number were in an aspirated condition. (Fig. 5, B.) In trees Nos. 4 and 5, which were heavily stained, 80 per cent or more of the tori were aspirated. There was very little difference be-

[7]) Scarth, G. W. The water content of heartwood in relation to wood structure. (Manuscript) Department of Botany, McGill University, 1931.

tween the tori of stained wood and of clean wood from an adjacent sector, which might indicate that there was a general, instead of a localized, drying in an infected tree. There is here no evidence to indicate whether the drying of the wood causes aspiration of tori or whether aspiration causes drying. The fact that the percentage of aspiration in adjacent sectors of stained and unstained wood is remarkably similar points to the



A. B.

Figure 5.
Tori in the bordered pits of shortleaf pine tracheids.
A. The median position. B. In aspirated condition.

possibility that aspiration follows drying. On the other hand, sectors of blued wood exert a decided influence on an unstained sector directly above.

A plausible explanation of the stoppage of water in bluestained areas is that aspiration of tori in blued wood is brought about by the action of the fungus, and that such areas more or less impervious to water eliminated to some degree the source of water supply to unstained sectors above. As a result these areas in some cases lose part of their water from the action of the transpiration pull from above, and desiccation is then followed by the aspiration of the tori.

An attacked tree, then, in its varying changing degrees of infestation and infection, is very complex. The sapwood consists of irregular areas of stained and unstained wood differing widely in their water content, the condition of their tori, and their permeability to water. Of the water relations or conditions existing in a tree we know very little; after attack there is a gradual diminution of water, however brought about, and eventually the tree dies.

Discussion.

The relative amount of damage caused by bark beetles and by blue-stain fungi in attacked pines is difficult to evaluate. Tunnels such as are made by the beetles cannot be simulated experimentally, nor can blue-stain be avoided in tunnels made by beetles. Ordinarily, pines attacked by the southern pine beetle and the accompanying fungus die within 3 or 4 weeks. The girdling caused by the galleries of the beetle is sufficient to cause death of infested trees in time, but it seems very unlikely that such girdling could cause death within a few weeks. The writer believes that the cause of death of pines attacked by *Dendroctonus frontalis* is the blue-stain fungus, *Ceratostomella pini*, which accompanies the beetle.

Experiments with ascent of dye in stained saplings and observations on inoculated trees indicate that areas of stained wood interfere with the transpiration current. If these regions of interference in the sapwood are so spaced that there is no continuous stain-free passage from the roots to the crown, the foliage dies from lack of water.

A number of hypotheses on the reason for water stoppage in such stained areas have been considered. The theory that hyphae are present in sufficient numbers to occlude the water-conducting elements is untenable because hyphae are confined very largely to the ray parenchyma cells and to the resin ducts. Hyphae in tracheids are not uncommon, but hyphae in numbers sufficent to cause occlusion have not been observed. Another explanation, that deleterious substances produced either by the fungi, the bark beetles, or both, were carried by the transpiration stream into the foliage with lethal results, is improbable because more than half of the sapwood in some inoculated trees was stained, yet the crowns remained alive if a continuous stain-free passage was present. Other trees with a much lesser proportion of stained sapwood died, but in such trees a complete cross-section was blued. If deleterious substances were produced by the fungus, the greatest reaction should presumably be found in those trees which had the greatest proportion of stained sapwood.

Another theory has suggested itself, and, although there is no experimental evidence to support it, it is mentioned as a possible explanation of stoppage in stained areas. Most physiologists agree that the presence of a gas bubble within a tracheid eliminates that tracheid from

the water-conducting system. In shortleaf pine 10 to 30 ray parenchyma cells adjoin each wood tracheid at different points along its length. It is conceivable that, because of the heavy fungus growth in the parenchyma cells of bluestained wood, gas, possibly carbon dioxide, evolved in the metabolic processes of the hyphae may be drawn into the tracheids and form bubbles. Although carbon dioxide is readily soluble in water, during periods of changing hydrostatic pressure the gas might pass out of solution in sufficient quantities to form bubbles within the tracheids. As the hyphae of the fungus gradually penetrate the sapwood along the medullary rays, increasingly greater numbers of tracheids may be eliminated from the hydrostatic system. Ultimately, so small an amount of water reaches the foliage that it dies from lack of water.

The most reasonable explanation, in the writer's opinion, is based on the belief that the action of *Ceratostomella pini* in the ray parenchyma cells brings about a permanent aspiration of tori in the water-conducting tracheids and stops the transpiration stream. This permanent condition of aspiration, as has been previously stated, might be brought about by the plugging of the perforations in the membranes of the bordered pits. Decomposition products of the fungi, or the protoplasmic residue of ray parenchyma cells attacked by these fungi, might be drawn into the transpiration stream in sufficient amounts to cause occlusion of the pits in the membranes. The tension of the water column might then cause a permanent deflection of tori from their median position.

Grosmann (2) in Germany has investigated the relation between bluestain fungi, yeasts, and bark beetles, and concluded that, although these micro-organisms are found in the alimentary tracts of the insects, they may not be necessary to the development of the beetles.

Even if bluestain fungi prove to be of no great significance in the nutrition of the southern pine beetle, the common association of the specific organism *Ceratostomella pini* with the insect leads to the conclusion that the association is something more than accidental. The fungi may be of decisive influence in altering the condition of the host tree necessary for brood development. St. George and Beal (9) stated that periods of abnormally dry weather favor the rapid development of broods of *D. frontalis* and that on the resumption of normal or heavy precipitation brood development is checked and the beetle is very nearly eliminated from the forest. If, as appears from the experiments, the bluestain reduces the water content in a tree, the development of the insect brood is no doubt favored by the presence of the stain. The southern pine beetle is able to make its tunnels and lay eggs in trees with high water content, but, in the writer's opinion, unless the staining fungi become established and reduce the water content of the wood, the brood is unable to develop. The fungus may thus be indispensable to the survival of the insect.

Summary.

1. The invariable presence of bluestain fungi in pines successfully attacked by bark beetles, and the phenomenon of specificity indicate that beetles are direct carriers of bluestain inoculum.

2. Bluestain fungi grow from points along galleries to form black patches of irregular shape and size on the surface of the wood. Hyphae also invade the sapwood along the medullary rays to form wedge-shaped sectors of blued wood.

3. Bluestain hyphae are largely confined to the medullary rays and resin ducts. They are found less commonly in the ray and wood tracheids.

4. Species of Ceratostomella have been isolated from bluestained wood and from different bark beetles. A cultural description of two species used in inoculation experiments is given.

5. Pines have been inoculated in a number of ways with species of Ceratostomella. Although results have been variable, many of the trees have become stained and some have died.

6. Moisture determinations made on normal trees indicate that the water content increases from the base to the top. In trees attacked by bark beetles and bluestain, the water content decreases from the base to the top.

7. The water content in stained regions in inoculated trees is much lower than in similar, but unstained, regions in check trees.

8. Experiments with stained and unstained saplings placed in dye show that dye will not pass through stained areas.

9. It is concluded that an infected tree is unable to draw water through the stained parts. If a cross-section of the stem is completely blued, or if there is no uninterrupted stain-free passage between the roots and crown, the tree dies from lack of water.

10. *Ceratostomella pini* is probably indispensable to the southern pine beetle, because the fungus brings about a reduction of the water content of infested trees to the degree necessary for beetle brood development.

11. Although the girdling effect of beetle tunnels would in time kill infested pines, it is believed that the cause of death is due to the action of bluestain fungi on the tori in the wood tracheids.

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Taschenatlas der Krankheiten des Weinstockes

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